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Nuclei Take a Position: Managing Nuclear Location

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Eukaryotic cells display considerable morphological plasticity linked to their abilities to carry out a myriad of complex functions. Structural rearrangements associated with cellular activities, from yeast mitosis to cell migration in the mammalian central nervous system, often involve relocation of the cell nucleus. Recent studies have provided insight into how nuclear components can be mechanically coupled to the cytoskeleton, providing a more complete understanding of the role of nuclear positioning in both health and disease.

Eukaryotic cells exhibit a wide assortment of specialized functions, both individually and as the building blocks of tissues. Implementation of many of these functions frequently involves rearrangement of cellular structures and polarized redistribution of organelles. In animal cells the nucleus is typically the largest organelle yet it is often repositioned, sometimes quite dramatically so, during the differentiation of diverse cell types including neurons, epithelial cells, and myocytes (Fridkin et al., 2008; Starr, 2009; Wilhelmsen et al., 2006). Nuclei are also actively positioned during mating, mitosis, and meiosis in fungi (Fridkin et al., 2008; Starr, 2009). Some of the mechanisms that direct this positioning have recently been identified, providing new insight into how the nucleus physically interacts with other cellular components. However, significant questions still remain concerning the orchestration of nuclear migration or positioning relative to other structures within the cell. Perhaps most important is to understand the ultimate role of nuclear migration and positioning in normal cell and tissue physiology.

Positioning Nuclei

It is possible to envisage both active and passive mechanisms for moving or locating nuclei within cells. In the passive scheme, cytoskeletal elements would be reorganized in such a way that the nucleus is simply displaced through restructuring of the cytoplasm. This same scheme could also be employed as a means of nuclear anchoring merely by restricting nuclear movement. The alternative active mechanism would involve the coupling of cytoskeletal components with the nucleus. Vectorial nuclear migration would be mediated by molecular motors that are associated, either directly or indirectly, with the nuclear surface. Similarly, nuclear anchoring would involve molecular tethers, most likely cytoskeletal elements, that are also linked to the cytoplasmic face of the nuclear envelope (NE). It is this second active mechanism that appears to represent a predominant means of nuclear positioning in most cell types (Starr, 2009).

Both actin- and microtubule-mediated nuclear positioning has been documented in a variety of organisms that include fungi (Morris et al., 1998a), plants (Ketelaar et al., 2002), and animals (Starr, 2009). In the case of the microtubule-based process, Reinsch and Gönczy (1998) have outlined two basic schemes by which this might occur. The first of these involves NE-anchored centrosomes or microtubule-organizing centers (MTOCs) with nuclear movement mediated either by astral microtubule polymerization or by engagement of astral microtubules with microtubule motor proteins anchored to other cellular structures (references in Reinsch and Gönczy, 1998). In this situation, the nucleus behaves essentially as a centrosomal passenger. Such movement is typical of the male pronucleus in fertilized *Xenopus* eggs. A similar situation is observed during mitosis in *S. cerevisiae* where migration of the nucleus into the bud neck results from tensile forces applied to the spindle pole body (the yeast equivalent of the MTOC) by microtubules whose tips are anchored within the bud cortex. Force generation in this case, however, appears to involve the depolymerization of these anchored microtubules (Adames and Cooper, 2000). This entire process is essential for the faithful distribution of chromosomes between mother and daughter yeast cells.

The second mechanism for microtubule-mediated nuclear migration depends upon the attachment of microtubule motor proteins such as cytoplasmic dynein to the nuclear surface. Accordingly, in this scheme the nucleus would represent a giant cargo that would track along microtubules. This appears to be the mechanism of female pronuclear movement in *Xenopus* eggs. Female pronuclear-associated dynein engages with astral microtubules focused at the male-derived centrosome causing the female pronucleus to move toward the male pronucleus (which itself is subject to MTOC-driven migration). This ultimately leads to pronuclear fusion. Evidence for such a scheme was provided by Reinsch and Karsenti (1997) who showed that nuclei assembled in vitro in *Xenopus* egg extracts had the capacity to migrate to the minus end of microtubules. Evidently this migration was mediated by cytoplasmic dynein anchored to the nuclear surface. Related studies in *C. elegans* embryos have also revealed a key role for cytoplasmic dynein, which includes a NE-associated dynein population, in both pronuclear migration and centrosome separation (Gonczy et al., 1999; Yoder and Han, 2001).

During the development of the vertebrate nervous system, there are two well-known, albeit poorly understood, examples of nuclear positioning that are at least partly dependent upon microtubules. Interkinetic nuclear migration (Sauer, 1935) is a feature of the pseudostratified columnar epithelial cells that form the neuroepithelium. These cells undergo asymmetric divisions to generate neurons for the central nervous system. As the neuroepithlial cell cycle progresses from G1 to S phase, the nuclei move from the apical to basal regions of the cell. After

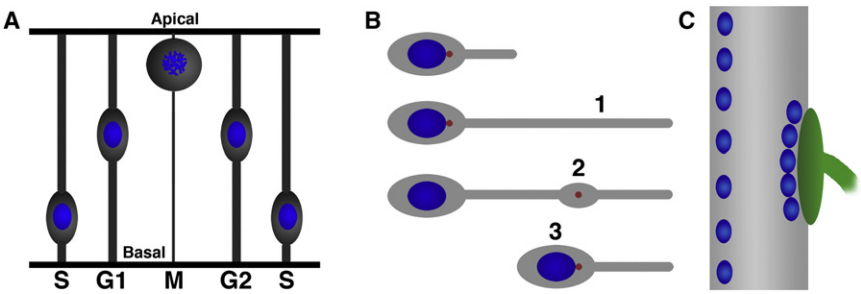

Figure 1. Examples of Nuclear Positioning
(A) The location of the nucleus during neuroepithelial interkinetic migration is correlated with cell cycle.
(B) During nucleokinesis, migrating neurons exhibit a three-step movement: (1) neurite extension followed by (2) MTOC migration, then (3) nuclear repositioning.
(C) In syncytial myofibers, distribution of most nuclei is row-like. A few, however, are clustered beneath the NMJ.

S phase the nucleus is returned to the apical domain in preparation for mitosis (Figure 1A). Throughout most of this back-and-forth migration, the apical and basal membranes remain extended, creating a bipolar morphology (Kosodo and Huttner, 2009). This complex series of events appears to regulate cell fate, either intrinsically through organelle distribution or extrinsically by interaction with neighboring cells in distinct regions of epithelium (Baye and Link, 2008).

Movement of newly formed neurons from the neuroepithelium depends upon the process of nucleokinesis (Figure 1B), the second example of nuclear migration in the nervous system. Neoroepithelial-derived neurons must travel several or even hundreds of cell lengths to their final destinations. The basic principle of neural migration is the extension of a process known as the leading neurite (Lambert de Rouvroit and Goffinet, 2001). Once the neurite is anchored, the nucleus follows along with the soma. As simple as this seems, the precise mechanisms behind these events remain unclear. First, the individual steps in nucleokinesis are not obviously synchronized. Second, the MTOC, which has been shown to precede the nucleus into the neurite, appears to play a critical role in nuclear movement (Tsai et al., 2007). However, the nucleus does not simply behave as a passenger of the MTOC as is seen for the male pronuclear movement. Whereas the MTOC seems to move smoothly in to the neurite, the nucleus follows in a series of jumps or saltations. In this way, the nucleus and MTOC may be periodically separated by 10–20 μm or more. MTOC movement into the neurite is dependent upon dynein and its regulator Lis1 (Dujardin et al., 2003), as is the movement of the nucleus. Indeed, Lis1 has been localized to the NE. However, the forward movement of the nucleus also requires actin and myosin II (Bellion et al., 2005; Schaar and McConnell, 2005; Tsai et al., 2007). Although the process of nucleokinesis is still not fully understood, most, but not all (Umeshima et al., 2007), studies indicate that it is the movement of the MTOC into the neurite that enables nuclear and hence neuronal migration.

A basic role for Lis1 and dynein in nuclear migration has been conserved through evolution. In the filamentous fungus *Aspergillus nidulans*, NUDF is the ortholog of Lis1 (Morris et al., 1998a). *A. nidulans* develops by extending linear syncytial mycelia into which nuclei migrate, with each nucleus apparently being pulled by its own spindle pole body (SPB), the functional equivalent of the centrosome. This migration is dependent upon both NUDF and dynein. The relationship between neuronal nucleokinesis and mycelial nuclear migration is further solidified by findings that an interaction between NUDF and NUDC, another protein required for nuclear migration in *A. nidulans* and which is localized in part to the SPB, is mirrored by an interaction between Lis1 and the mammalian ortholog of NUDC (Helmstaedt et al., 2008; Morris et al., 1998b).

It is clear that in many situations, nuclear positioning and MTOC positioning are inextricably linked. However, understanding the role that either organelle plays in the positioning of the other is bedeviled by questions both of positional relativism and timing (which organelle is moving and when is it being moved?). For example, in neurons it is the MTOC that appears to move first toward the neurite extension followed later by the nucleus (Solecki et al., 2004). However, in the initiation of fibroblast migration in vitro, it is the nucleus that has been proposed to first move rearward of the MTOC that itself remains immobile (Gomes et al., 2005). What is also unclear is whether any general rule exists concerning the orientation of the nucleus relative to the MTOC in migrating cells. This has typically been followed in scratch or wound healing assays in tissue culture systems. In many cell lines the MTOC is usually oriented toward the wound edge, in front of the nucleus (Gomes et al., 2005). However, this is not universally true for all cells or in all circumstances (Danowski et al., 2001; Yvon et al., 2002). For instance, if cells are allowed to migrate on a spatially constrained extracellular matrix substrate, then it appears that the nucleus can be positioned toward the front of the cell, sometimes far ahead (as much as 10–20 μm) of the MTOC and associated Golgi apparatus (Pouthas et al., 2008).

The Nuclear Envelope

The organization of the nuclear envelope imposes certain constraints on how effective force-generating components of the cytoskeleton can be coupled to the nuclear surface. The global structure of the NE has been highly conserved through evolution (Gruenbaum et al., 2005; Stewart et al., 2007). In all cell types, the NE features prominent inner and outer membranes (INM and ONM) separated by a 20–50 nm gap or perinuclear space (PNS). As a general rule, the ONM is continuous with and forms part of the endoplasmic reticulum. It is invariably studded with ribosomes and is active in the synthesis of membrane and secretory proteins. The INM in contrast is ribosome-free and contains a unique spectrum of integral membrane proteins, at least 50–60 of which have been identified (Schirmer et al., 2003). Despite their biochemical differences, the INM and ONM are connected at annular junctions that form aqueous channels between the nucleoplasm and cytoplasm, and which are occupied by nuclear pore complexes (NPCs) (Terry et al., 2007). These highly elaborate structures mediate the regulated movement of macromolecules between the

nucleus and cytoplasm. It follows that the INM, ONM, and ER constitute a single continuous membrane system, with the PNS forming a perinuclear extension of the ER lumen.

In metazoans, an additional nuclear structural component, the nuclear lamina, is evident (Gruenbaum et al., 2005; Rowat et al., 2008). The nuclear lamina consists of a thin (~10–50 nm) highly insoluble protein meshwork that is intimately associated with both the nuclear face of the INM and the underlying chromatin. In addition, the lamina functions as an attachment site for NPCs. The major components of the nuclear lamina are the A- and B-type nuclear lamins, a group of type-V intermediate filament proteins. Like their cytoplasmic counterparts, the lamins feature a central coiled-coil domain flanked by nonhelical head and tail domains and have the capacity to assemble into nonpolar filaments. Certainly in *Xenopus* oocyte NEs the single major nuclear lamin seems to be organized in arrays of 10 nm intermediate-like filaments (Aebi et al., 1986; Goldberg et al., 2008). Whether the same is true in vertebrate somatic cells, which may contain 3–4 lamin species, is unclear. Both the A- and B-type lamins have been shown to interact with multiple chromatin and INM proteins. In this way the lamina may play an important role in anchoring chromatin domains at the nuclear periphery.

Mutation of the major B-type lamin in *Drosophila* provided the first confirmation of the notion that the lamina plays an essential role in the maintenance of NE integrity (Lenz-Böhme et al., 1997). The recognition that multiple human diseases such as muscular dystrophy, progeria, lipodystrophy, and leukodystrophy are linked to mutations in the genes encoding both A- and B-type lamins, and which are frequently associated with nuclear dysmorphology, only served to reinforce this view (Worman and Bonne, 2007; Daur and Worman, 2009 [this issue]). Disruption of the lamin A gene (*Lmna*) in mice by homologous recombination is also associated with nuclear and NE structural abnormalities and provided compelling evidence that the localization of certain INM proteins, emerin for example, was dependent in part on interactions with the lamina (Sullivan et al., 1999). More surprisingly, biomechanical studies on $Lmna^{-/-}$ mouse embryo fibroblasts (MEFs) revealed that they have a less resilient and more deformable cytoskeleton than their $Lmna^{+/+}$ counterparts (Broers et al., 2004; Hale et al., 2008; Lammerding et al., 2004, 2006; Lee et al., 2007). One of the more subtle changes in the cytoarchitecture of fibroblastic cells that is associated with deficiencies in either A-type lamins or emerin is the dissociation of the MTOC from the nuclear periphery (Houben et al., 2009; Lee et al., 2007). Hutchison and colleagues have suggested that the usual linkage between the NE and MTOC might be mediated by a small population of emerin in the ONM (Salpingidou et al., 2007). $Lmna^{-/-}$ cells also display defective induction of mechanosensitive genes and reduced resistance to mechanical stress (Lammerding et al., 2004). Taken together, these data indicate that there has to be some form of communication or coupling between the nuclear lamina and the cytoskeleton. The nature of this communication is now being revealed in studies on both the microtubule- and actin-based migration of nuclei in a variety of systems.

Nucleo-cytoplasmic Coupling

Allan and Vale (1994) demonstrated that ER tubules can extend along microtubules in a dynein-dependent manner. Obviously the ER must contain dynein binding partners for this to occur. The ONM is an extension of the ER, so an obvious question is whether these same dynein-binding proteins might be responsible for dynein- and microtubule-based nuclear migration. Reinsch and Gönczy (1998) argued that this is probably not the case because there would be nothing to prevent the dynein-driven emanation of membrane tubules from the ONM. Instead, they suggest that there must be a mechanism to mechanically couple an ONM dynein-binding protein to the INM or even to the lamina or other nuclear components. To put it simply, there must be a mechanism to transmit force across the entire NE.

The existence of such a force transmission mechanism within the NE had also been proposed by Ingber and colleagues (Maniotis et al., 1997). They used RGD peptide or fibronectin-coated beads to bind integrins on the surface of cultured fibroblasts. Subsequent displacement of the beads with a microneedle led to integrin-mediated deformation of both the cytoskeleton and the nucleus. Furthermore, the nuclear content was also displaced in the direction of bead movement. The implication of this experiment is that there has to be a mechanism for connecting the cytoskeleton (which interacts with integrins at the cell surface) to the nuclear contents. In other words there has to be some form of link that is able to span both nuclear membranes (Wang et al., 2009). Whereas NPCs could fit the bill for such linkages, being exposed to both the cytoplasmic and nuclear environments as well as being anchored to the nuclear lamina, their role in force transmission is uncertain. Instead, there is a growing body of evidence that proteins of both the INM and ONM can interact across the PNS and which may then couple nuclear and cytoskeletal components. We now refer to such translumenal protein assemblies as LINC complexes (for linker of the nucleoskeleton and cytoskeleton).

SUN and KASH Proteins

The molecular basis for the mechanical coupling of nuclear and cytoplasmic structures and the definition of LINC complexes began to emerge from studies on Unc-83, a *C. elegans* nuclear membrane protein. Unc-83 is required for microtubule-dependent nuclear migration in a variety of cell types. Together with Unc-84, an INM protein with which it was shown to interact, Unc-83 was proposed to mediate force transmission across the NE. Subsequent analyses of Anc-1, a very large ONM protein (Starr and Han, 2002) that is required for the anchorage of nuclei within the *C. elegans* hypodermal syncytium, suggested how such force transmission might be accomplished. Anc-1 contains a single transmembrane domain close to the C terminus and a small lumenal domain of about 40 amino acid residues. The bulk of the molecule resides within the cytoplasm and features an extended series of helical repeats and an N-terminal actin binding domain (ABD). The C-terminal region of Anc-1, consisting of the transmembrane and lumenal domains was found to be conserved in several other ONM proteins, notably *Drosophila* Klarsicht (Mosley-Bishop et al., 1999) and mammalian Syne-1 (also known as nesprin-1, Myne-1, and ENAPTIN) (Apel et al., 2000; Mislow et al., 2002b; Padmakumar et al., 2004). This conserved region is commonly referred to as a KASH domain (Klarsicht, Anc-1, Syne-1 homology) (Starr and Han, 2002). Unc-83, now known to reside in the ONM, is also a member of the KASH domain protein family (Figure 2, Table 1).

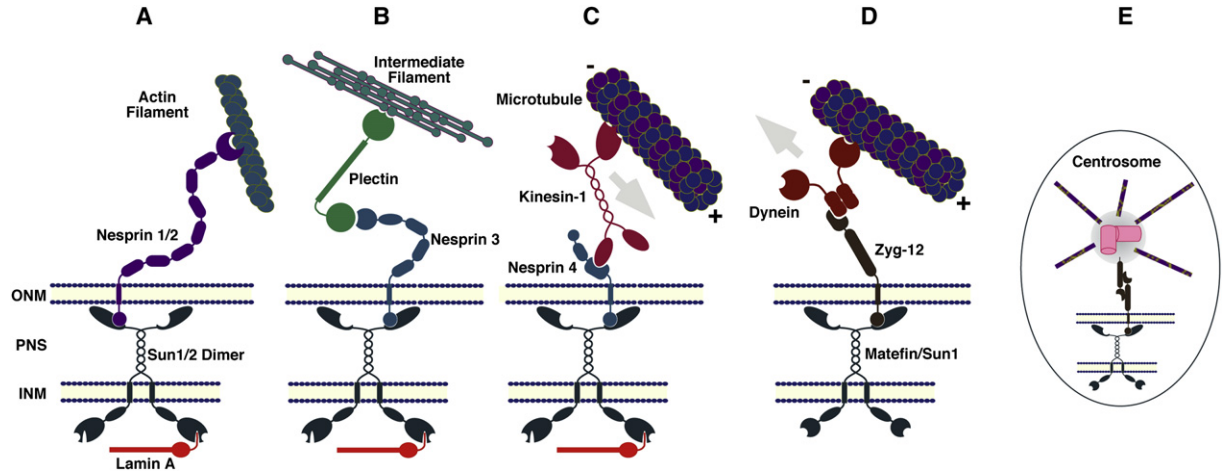

Figure 2. The LINC Complex Connects Nuclear Structures to the Cytoskeleton

INM SUN-domain proteins function as transluminal tethers for ONM KASH-domain proteins. The nucleoplasmic domain of SUN proteins binds to lamins and/or other nuclear components. Cytoplasmic domains of nesprins interact with cytoskeletal elements, including actin, plectin, and kinesin. Single examples of cytoskeletal interactions are provided in (A)–(D). The models displayed in (A)–(C) are of mammalian SUN and KASH domain proteins. Sun1/matefin and Zyg-12 portrayed in (D) are *C. elegans* proteins. A more comprehensive summary of KASH protein function and interactions is provided in Table 1. (E) presents a model of centrosome anchoring involving homotypic interactions between NE- and centrosome-associated isoforms of Zyg-12 in *C. elegans* (Malone et al., 2003). Nesprin 3 (B) is known to form higher-order oligomers. However, for simplicity it is depicted as a monomer.

Localization of Anc-1, Unc-83, or indeed any other KASH domain protein to the ONM begs the question of what prevents them from simply drifting off in to the peripheral ER. Studies in *C. elegans* revealed that retention of both Unc-83 and Anc-1 in the ONM is contingent upon the INM protein Unc-84 (Starr and Han, 2002). The C terminus of Unc-84 resides within the PNS and contains a ~200 amino acid residue sequence that is found in a number of other NE proteins, including Sad1, a component of the *S. pombe* spindle pole body (Hagan and Yanagida, 1995). This conserved sequence is termed a SUN domain (Sad1p, Unc-84) (Malone et al., 1999). Localization of Unc-84 itself is in turn dependent upon the single *C. elegans* lamin (Lee et al., 2002). Taken together, these observations led to the proposal by Starr and Han (2003) and by Lee et al. (2002) that Unc-84 in the INM might function as a translumenal tether for Anc-1 in the ONM. If this were to be the case, then Anc-1 and Unc-84 would represent a pair of links in a molecular chain that connects the actin cytoskeleton to the nuclear lamina. Similarly, Unc-83 together with Unc-84 would provide an alternative connection between the lamina and the microtubule system (McGee et al., 2006). As an emerging theme then, SUN proteins in the INM would function as tethers for KASH proteins in the ONM, and together these KASH-SUN pairs would represent the core elements of LINC complexes (Figure 2).

Table 1. Function and Interactions of KASH Domain Proteins

Cytoskeletal Association	KASH Protein	Organism	Function	Phenotypes of KASH Protein Mutants
Actin	Anc-1	*C. elegans*	nuclear anchoring in syncytial hypodermal cells	reduced brood size, pale and thin appearance
	Msp-300	*Drosophila*	unclear	larval lethal
	Nesprin-1	mouse	nuclear anchorage at muscle NMJ	inconsistent phenotypes including no effect, embryonic lethal, or EDMD-like
	Nesprin-2	mouse	none reported	none reported
Plectin and IF-system	Nesprin-3	mouse	none reported	none reported
Kinesin	Unc-83	*C. elegans*	nuclear migration in P-cells, hyp7 cells, and intestinal primordial cells	uncoordinated movement with defective egg-laying
	Nesprin-4	mouse	none reported	none reported
Dynein	Zyg-12	*C. elegans*	nuclear-centrosome association in embryonic and germ cells	embryonic lethal
	Klarsicht	*Drosophila*	nuclear positioning in developing eye	aberrant eye morphology
	Kms1	*S. pombe*	meiotic bouquet formation and karyogamy	aberrant meiotic recombination and chromosome segregation, failed karyogamy

References are contained in the text.

LINC Complex Assembly

The question of whether KASH domain proteins in the ONM are directly tethered by INM SUN domain proteins was first addressed experimentally in mammalian cells. Four mammalian KASH domain proteins have been described to date (Figure 2; Table 1). The first of these, Syne1 (also known as nesprin1, Myne1, and ENAPTIN) was originally identified in muscle (Apel et al., 2000; Mislow et al., 2002b). Muscle cells develop as syncytia with the majority of their nuclei arranged, often row-like, just beneath the plasma membrane. A few nuclei, however, are invariably found clustered beneath the neuromuscular junction (NMJ; Figure 1C). It is in the NEs of these nuclei that Syne1/Nesp1 was found to be enriched (Apel et al., 2000). The suggestion was that Syne1/Nesp1 could be required for the clustering of these nuclei, which may be specialized for the transcription of NMJ-specific genes.

The Syne1/Nesp1 gene encodes a plethora of splice isoforms, the largest of which is 1000 kDa (Apel et al., 2000; Mislow et al., 2002b; Padmakumar et al., 2004; Zhang et al., 2001, 2002). This immense protein, often referred to as nesprin 1 Giant (Nesp1G), like Anc-1, features an N-terminal actin binding domain (ABD) consisting of paired of calponin homology domains. The ADB is followed by multiple spectrin repeats, suggesting that Nesp1G is a highly flexible molecule. Like Anc-1, Nesp1G is anchored in the ONM by a C-terminal KASH domain. The nesprin 2 (also known as Syne2 and NUANCE) gene also encodes a giant isoform (Nesp2G) of about 800 kDa that conforms to the same overall structure as Nesp1G including an N-terminal ABD, spectrin repeats, and KASH domain (Apel et al., 2000; Zhang et al., 2001, 2002; Zhen et al., 2002). Both Nesp1G and Nesp2G appear to be widely expressed. Although Nesp1G and Nesp2G seem to be restricted to the ONM, other smaller isoforms may have access to the INM where it has been shown that they may interact with lamins and INM proteins such as emerin (Mislow et al., 2002a; Wheeler et al., 2007; Zhang et al., 2005).

The KASH domains of Nesp1G and Nesp2G are necessary and sufficient for localization to the ONM (Zhang et al., 2001). Because overexpression of the KASH domain of one will displace the other from the INM, it is likely that Nesp1G and Nesp2G share a common localization mechanism (Grady et al., 2005; Zhang et al., 2007). Mammalian somatic cells contain two SUN domain proteins, Sun1 and Sun2 (Malone et al., 1999). Both of these proteins (including several splice isoforms) are widely expressed and are localized exclusively to the INM. Although both Sun1 and Sun2 bind A-type lamins, this interaction is not essential for their appropriate localization, at least in certain cell types (Crisp et al., 2006; Haque et al., 2006; Hasan et al., 2006). It is likely that these two proteins may associate with a variety of other NE and chromatin components. Indeed, Sun1 is known to bind hALP, a human membrane-associated histone acetyltransferase (Chi et al., 2007). If, by analogy with Unc-84 and Anc-1, Sun1 and Sun2 were involved in nesprin localization, then depletion of both SUN domain proteins should lead to loss of nesprins from the ONM. Indeed, this is exactly what occurs (Crisp et al., 2006; Padmakumar et al., 2005). Furthermore, expression of a dominant-negative form of Sun1, consisting of the soluble lumenal domain (which includes the SUN domain) targeted to the PNS and ER lumen, evicts Nesp2G from the NE (Crisp et al., 2006). Expression of this Sun1 mutant also leads to the separation of the INM and ONM and dilation of the PNS (Crisp et al., 2006). The same effect can be achieved through depletion of Sun1 and Sun2 by RNA interference (Crisp et al., 2006). Evidently the SUN proteins have an important role in the maintenance of the regular spacing of the INM and ONM.

Taken together, these data strongly suggest that the Sun1/2 lumenal domain and the nesprin KASH domain interact across the PNS. This suggestion has been born out in a series of coimmunoprecipitation studies utilizing either in vitro translated SUN and KASH proteins or SUN proteins and nesprins expressed in tissue culture cells (Crisp et al., 2006; Haque et al., 2006; Padmakumar et al., 2005). The conclusion is that Sun1/2 and nesprins form complexes that span both nuclear membranes and in this way are able to couple nuclear components, including nuclear lamins, with elements of the cytoskeleton (Figure 2). In the case of Nesp1G and Nesp2G, this would involve the actin system. Given the association between the actin cytoskeleton and cell surface integrins, the interaction of Nesp1G and Nesp2G with Sun1/2 in LINC complexes might mediate the mechanical coupling of nuclear components with the plasma membrane. In this way, LINC complexes containing Nesp1G and Nesp2G may provide a molecular basis for the vectorial nuclear distortion after the physical displacement of integrins observed by Maniotis et al. (1997).

Nesprins and Nuclear Positioning

Given its preferential localization in the membranes of nuclei residing beneath the postsynaptic membrane of myofibers, does Nesp1G have a role in clustering nuclei in this location? This has been addressed in two ways. The first was to overexpress a nesprin1 KASH domain in muscle cells in transgenic mice (Grady et al., 2005). The expectation was that this KASH domain should displace endogenous Nesp1G from the NE. The second approach was to disrupt the Nesp1 gene in mice by homologous recombination in such a way that the KASH domain was eliminated (Zhang et al., 2007). In both systems, immunofluorescence analyses revealed a decline in the number of nuclei in the postsynaptic clusters. Evidently, Nesp1G is indeed involved in recruiting nuclei to, or anchoring nuclei at, the NMJ. Intriguingly, loss of postsynaptic nuclei had no discernible effect on muscle function or innervation in these studies. Similarly, mice harboring homozygous KASH-less disruptions of the Nesp2 gene displayed no overt abnormalities. However, loss of both Nesp1 and Nesp2 was found to be perinatal lethal with death seemingly linked to respiratory failure, possibly the result of diaphragm dysfunction (Zhang et al., 2007).

The conclusion to be drawn here is that nesprins 1 and 2 are to a large extent functionally redundant. These findings are, however, complicated by a more recent study in which mice homozygous for a similar KASH-less form of nesprin 1 were found to be severely compromised with 50% dying at birth, apparently because of respiratory failure. The survivors were subsequently observed to develop muscular dystrophy (Puckelwartz et al., 2009). This finding would actually be consistent with recent suggestions that some forms of autosomal Emery Dreifus muscular dystrophy (EDMD), a disorder normally associated with mutations in the lamin A gene, may be caused by nesprin defects. As an intriguing side bar to this, two mouse models of *Lmna*-linked EDMD feature NMJ abnormalities and an inability

to cluster nuclei beneath the postsynaptic membrane (Méjat et al., 2009). This is suggested to be due in part to a loss of Sun2 from the NE with a consequent failure to retain Nesp1G in the ONM. However, observations on both Sun1 and Sun2 knockout mice indicate that these two proteins share overlapping functions in Nesp1G retention as well as anchoring of NMJ nuclei. Indeed, mice deficient in both Sun1 and Sun2 display perinatal mortality reminiscent of that observed in Nesp1/2 double knockout animals (Lei et al., 2009).

One other actin-binding KASH protein has been described: Msp-300 in *Drosophila* (Starr and Han, 2002; Volk, 1992). Like the mammalian nesprins, Msp-300 features several spectrin repeats within its large cytoplasmic domain and an actin binding site close to its N terminus. Msp-300 is expressed both in muscle and in ovarian nurse cells. The precise function of Msp-300 in these tissues is still uncertain. A mutation in Msp-300 (*msp-300^{SZ-75}*) was originally found to cause larval lethality linked to defective myogenesis (Rosenberg-Hasson et al., 1996). However, it is not clear that muscle-specific isoforms of Msp-300 actually contain the C-terminal KASH domain. Consequently they are unlikely to be NE associated.

Correct nuclear positioning in nurse cells is a prerequisite for normal oogenesis. During this process, cytoplasm from the nurse cells is discharged (or "dumped") into the developing oocyte via specialized channels or ring canals in the apposed plasma membranes. Actin-dependent anchoring of nurse cell nuclei prevents them from blocking the ring canals and interfering with dumping. Whereas KASH-containing isoforms of Msp-300 are localized to the nurse cell NE (Yu et al., 2006), recent data from several laboratories have ruled out an essential role in nuclear anchoring (Technau and Roth, 2008; Xie and Fischer, 2008). Expression in the *Drosophila* germline of a mutant form of Msp-300 that lacks the KASH domain has no effect on nurse cell nucleus localization. Deletion of Klaroid, the single *Drosophila* SUN domain protein, also has no effect on nuclear positioning in nurse cells. It does, however, lead to the loss of Msp-300 from the NE, suggesting that Klaroid and Msp-300 associate as a *Drosophila* LINC complex isoform. As to the function of Msp-300 in muscle and ovaries, this is still an open question. Evidently, nurse cells possess other as yet unknown mechanisms involved in nuclear anchoring.

LINCs to the Intermediate Filament System

The third mammalian KASH domain family member, Nesprin 3 (Nesp3), like Nesprins 1 and 2, is targeted to the ONM (Wilhelmsen et al., 2005). Localization of Nesp3 to the ONM is dependent upon Sun1 and Sun2 in the INM, and not surprisingly overexpression of Nesp3 (or the Nesp3 KASH domain) will displace other nesprins from the NE and vice versa (Ketema et al., 2007; Stewart-Hutchinson et al., 2008). Thus Nesp3, which is widely expressed, defines an additional isoform of the mammalian LINC complex. Nesp3 is unusual among the KASH domain proteins that have been identified to date in that it binds plectin, a large (500 kDa) bifunctional cytolinker that can provide a connection to the intermediate filament system (Ketema et al., 2007; Wilhelmsen et al., 2005). Compared with giant nesprins, Anc-1 and Msp-300, Nesp3 is relatively small at about 110 kDa. However, it still contains multiple spectrin repeats and forms homodimers (Wilhelmsen et al., 2006). Plectin, also a parallel homodimer, binds to the N-terminal region of Nesp3 via its own N-terminal actin-binding domains. Plectin itself is an extended molecule, so the combined Nesp3-plectin pair would be comparable in size to Nesp2G. In addition to Nesp3, the plectin ABDs may also bind to the cytoplasmic tail of the integrin β4 subunit at the cell surface (Wilhelmsen et al., 2006). By providing additional links to IFs via its paired C termini, plectin may then mediate the coupling of cell surface and extracellular matrix structures to the Nesp3 LINC complexes of the nuclear envelope (Wilhelmsen et al., 2006). In this way, Nesp3 and plectin could contribute to the type of nuclear deformation observed by Maniotis et al. (1997) upon displacement of cell surface molecules.

Liu et al. (2007) recently reported that mammalian Sun1 and Sun2, although displaying some functional redundancy, are largely segregated in the plane of the INM. In particular, Sun1 tends to be concentrated around NPCs. Consequently, one might predict that a proportion of the nesprins, including Nesp3, would be tethered in the immediate vicinity of NPCs by Sun1. This, then, could provide an explanation for ultrastructural observations that some IFs appear to terminate at or associate with the cytoplasmic face of NPCs (Goldman et al., 1985).

Microtubules and Dynein at the NE

As discussed above, microtubule-based positioning of the nucleus to a large extent goes hand in hand with MTOC positioning. This is exemplified by the movement of nuclei toward the apical region of photoreceptors during eye development in *Drosophila*. This movement requires Klarsicht, a prototype KASH domain protein that binds cytoplasmic dynein (Mosley-Bishop et al., 1999). The recruitment of dynein to the ONM by Klarsicht serves to maintain a close association between the nucleus and the MTOC (Patterson et al., 2004). In Klarsicht mutants, the nuclei and MTOC separate with the MTOC alone, moving toward the photoreceptor apex while the nucleus becomes localized within the basal region of the cell. Evidently the nucleus would normally be a passenger on the MTOC. Localization of Klarsicht to the ONM is dependent upon the *Drosophila* lamin Dm_0 and the SUN domain protein Klaroid (Kracklauer et al., 2007). The obvious conclusion is that Klarsicht is tethered in the ONM via a translumenal interaction with Klaroid, which in turn is retained in the INM through binding to the lamin. Clearly Klarsicht and Klaroid, like other KASH/SUN protein pairs, conform to the LINC complex paradigm. It is not surprising, therefore, that Klaroid mutant flies display much the same rough eye phenotype that is observed in Klarsicht mutants (Kracklauer et al., 2007). Klarsicht has an additional role in lipid droplet transport in *Drosophila* embryos (Welte et al., 1998). This function of Klarsicht, however, is independent of Klaroid. It involves an isoform (Klarsicht β) that lacks the KASH domain and instead contains an alternative C terminus that confers targeting to lipid droplets rather than to the ONM (Guo et al., 2005).

A dynein-binding KASH domain protein has also been identified in *C. elegans*. Zyg-12, an 80–90 kDa protein that is synthesized as several splice isoforms, is related to members of the mammalian Hook protein family (Malone et al., 2003). Hook proteins are proposed to function as linkers between organelles and microtubules. In *C. elegans*, Zyg-12 has been shown to play an essential role in nuclear positioning in the gonad as well as in pronuclear migration in the early embryo (Malone

et al., 2003; Zhou et al., 2009). Normally, the *C. elegans* sperm introduces the only centrosome or MTOC into the egg. After centrosome duplication, the daughter centrosome becomes associated with the male pronucleus but migrates away from the original centrosome to the opposite nuclear pole. The ability of the daughter centrosome to attach to the male pronucleus appears to be strongly influenced by nuclear surface area and the accessibility of ONM-associated dynein (Meyerzon et al., 2009). It is suggested that the female pronucleus moves toward the male pronucleus along centrosomal microtubules. This ultimately leads to pronuclear fusion and is immediately followed by the first mitotic division. In the absence of functional Zyg-12, centrosomes become separated from the male pronucleus and pronuclear fusion fails to occur, resulting in aberrant mitosis and missegregation of the chromatids (Malone et al., 2003). Zyg-12 is tethered in the ONM via interactions with Sun1/matefin in the INM (Malone et al., 2003; Penkner et al., 2007). Because the cytoplasmic domain of Zyg-12 binds dynein, this provides a means to reel in centrosomes to the NE, by taking advantage of the dynein minus end motor activity. A soluble KASH-less isoform of Zyg-12 (Zyg-12A) is also found at the centrosome (Malone et al., 2003). Homotypic binding interactions between NE and centrosomal forms of Zyg-12 then stabilize the association of the centrosome with the NE. Surprisingly, in the *C. elegans* gonad, nuclear anchoring is centrosome independent. Whereas the centrosome itself is NE associated, nuclear anchoring is mediated by the engagement of Zyg-12-associated dynein with noncentrosomal microtubules that are nucleated at the plasma membrane (Zhou et al., 2009).

Kinesin at the NE

In mammals, a fourth nesprin, Nesp4, has recently been identified that has the capacity to bind the plus end motor protein Kinesin 1 (Roux et al., 2009). Nesp4 is a mere 42 kDa with only a single spectrin repeat. It is targeted to the ONM by its somewhat degenerate KASH domain and retained by virtue of interactions with Sun1 or Sun2 in the INM. In this way, Nesp4 defines yet another mammalian LINC complex isoform. Whereas nesprins 1–3 are widely expressed, Nesp4 is unusual in that it is found almost exclusively in secretory epithelial cells. As a rule, epithelial cells feature noncentrosomal microtubules that are arranged in lateral bundles with their plus ends oriented toward the base of the cell (Bacallao et al., 1989). Although the function of Nesp4 is uncertain, its capacity to bind Kinesin 1, which in turn might engage with the lateral microtubules, suggests that it could have a role in the positioning of the nucleus close to the basal membrane (Figure 3). When ectopically expressed in nonpolarized cells containing a centrosomally focused microtubule array, the presence of Nesp4 on the NE results in the dramatic separation of the nucleus from the centrosome, often by 20 μm or more (Figure 3). This can be easily explained by the plus end motor activity of kinesin, recruited to the NE by Nesp4, driving the nucleus away from the centrosome (or vice versa; Figure 3). These findings suggest that Nesp4 might contribute to the apical migration of the centrosome and Golgi apparatus that occurs during epithelial morphogenesis.

The *C. elegans* KASH domain protein, Unc-83, was originally implicated in microtubule-dependent nuclear migration and positioning in a variety of cell types (Starr et al., 2001). Localization of Unc-83 to the ONM is dependent upon the INM SUN protein Unc-84 (Starr et al., 2001), with which it shares a translumenal interaction (McGee et al., 2006). Recent studies have revealed that the cytoplasmic domain of Unc-83 binds kinesin-1. In this way, Unc-83 functions as a NE cargo adaptor for kinesin and together with Unc-84 defines a *C. elegans* LINC complex isoform that mediates microtubule association with the nucleus.

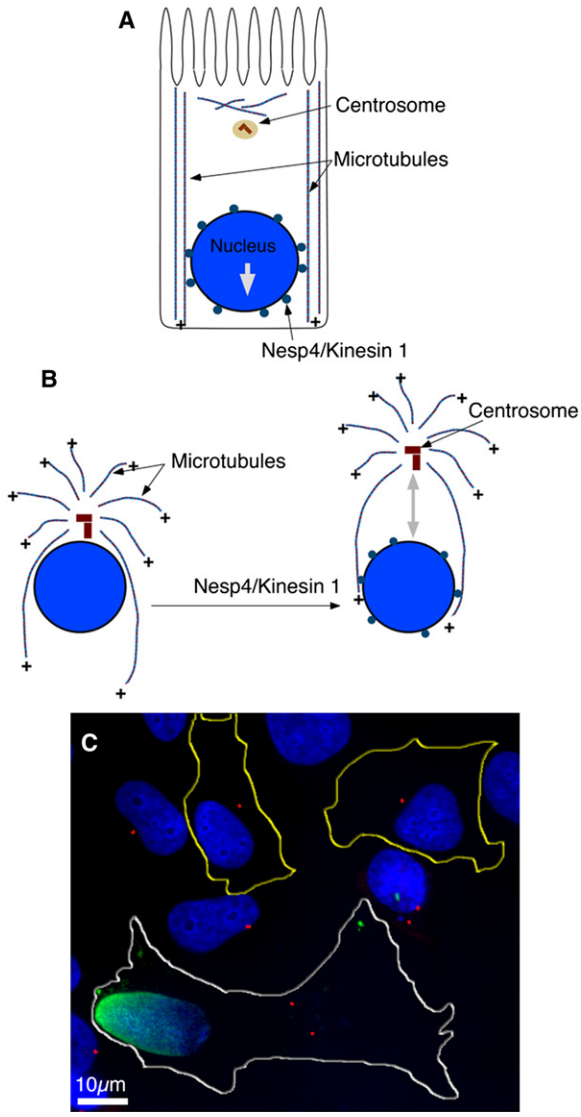

Figure 3. Nesp4 Actively Displaces the Nucleus from the MTOC in Epithelial Cells
(A) Polarized epithelial cells feature lateral bundles of microtubules with their minus ends oriented toward the apical membrane, a basal nucleus, and apical centrosomes and golgi apparatus.
(B) Expression of kinesin-binding Nesp4 in a cell with a fibroblast-like organization would be predicted to induce separation of the nucleus and MTOC.
(C) Expression of GFP-nesprin-4 (green) in HeLa cells causes the nucleus (blue) and MTOC (red) to move apart. Nesp4 often concentrates toward the pole of the nucleus furthest from the MTOC.

The LINC Complex in Nuclear Dynamics

Some of the same mechanisms that regulate movement of the entire nucleus can also rearrange intranuclear constituents

(Chikashige et al., 2007). We have seen that LINC complex isoforms mediate both nuclear migration and anchoring in a broad range of cell types from both vertebrates and invertebrates. A key property of LINC complexes is that they have the capacity to transmit forces from the cytoskeleton across the NE to the nuclear lamina and chromatin components. It should come as no surprise, then, that there is a growing recognition that LINC complexes may mediate both actin- and microtubule-based movement of chromosomes. This is particularly evident during meiotic prophase in both single-cell organisms and metazoa, and is described exhaustively elsewhere (Fridkin et al., 2008; Hiraoka and Dernberg, 2009 [this issue]).

Could LINC complexes contribute to the nuclear structural rearrangements that occur during mitosis in metazoa? It has been demonstrated that ONM-associated dynein engaged with astral microtubules facilitates NE breakdown by effectively peeling open the nuclear membranes (Beaudouin et al., 2002; Salina et al., 2002). Recent studies on neural stem cells indicate that the dynein regulator Lis1 is also involved in this process (Hebbar et al., 2008). However, the identity of the dynein binding partner on the nuclear surface has yet to be revealed. Furthermore, there has been a paucity of data on the fate of the LINC complex during mitosis, beyond the differential localization of Sun proteins during NE reassembly (Liu et al., 2007; Wang et al., 2006). Similarly, little is known about the regulation of SUN-KASH interactions. However, there is speculation that Torsin A (Breakefield et al., 2001), a AAA-ATPase family member and ER-resident protein, could have a role. Defects in Torsin A have been linked to the neurological disorder DYT1 dystonia (Ozelius et al., 1999). There is good evidence that Torsin A has at least one nuclear membrane binding partner because DYT1-linked Torsin A mutants concentrate in the PNS (Goodchild and Dauer, 2004, 2005). Furthermore, there are indications that Torsin A may interact with LINC complex components and displace both Sun2 and nesprins from the NE (Nery et al., 2008; Vander Heyden et al., 2009). Such an effect might account for the finding that Torsin A null MEFs display delayed migration and aberrant nuclear reorientation in wound healing assays (Nery et al., 2008). Given the role of AAA-ATPases in modulating protein-protein interactions, the notion that Torsin A might contribute to the regulation of translumenal SUN-KASH associations remains an attractive possibility.

Future Positions

The last few years have witnessed important advances in our understanding of the mechanisms of nuclear positioning. This has arisen through studies on a broad range of organisms, from yeast to humans, which have underscored the early evolutionary origins of this process. The identification of molecules that mediate both nuclear migration and anchoring has revealed how mechanical forces can be transmitted across the NE and suggests novel pathways for mechanotransduction. The ectopic expression of a single ONM protein, Nesp4, can induce dramatic changes in cellular organization. The implication is that the differential expression of certain LINC complex isoforms might have an important role in defining both cell and tissue architecture. This issue will certainly be addressed in the years to come in a variety of model organisms as well as in tissue-culture systems. The role of LINC complexes in the NE also provides a potential basis for the changes in cytoskeletal mechanics that have been observed in cells deficient in A-type lamins or in emerin. The inference is that KASH and SUN proteins may have a direct role in the etiology of laminopathies such as Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy. Similarly, the regulation of SUN-KASH interactions is likely to receive significantly more scrutiny given the potential role for Torsin A in this process and its links to human disease. Although we know that SUN proteins do bind A-type lamins, there is little doubt that they must also interact with other nuclear proteins. It is likely, therefore, that we have barely begun to appreciate the range of nucleocytoplasmic connections that are mediated by LINC complexes.

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